Thermal Resistance of *Listeria monocytogenes* and *Salmonella* spp. in Liquid Egg White

ABSTRACT

Survival of a five-strain mixture of *Listeria monocytogenes* and a six-strain mixture of *Salmonella enteritidis, S. typhimurium,* and *S. senftenberg* (not 775W) in liquid egg white was determined by a submerged-vial technique at 51.5°C and 53.2°C with 0.875% added H_2O_2 and at 55.5°C, 56.6°C, and 57.7°C with no additions. Survival at a range of pH values at 56.6°C also was determined. Surviving bacteria were counted on tryptic soy agar and results expressed as D-values; log-unit reductions in counts in 3.5 min or 6.2 min were calculated from these D-values. Plate pasteurization of commercially broken egg white (pH 8.8) inoculated with a single strain of *L. innocua* or *S. senftenberg* also was performed. Heating under currently approved pasteurization conditions, 51.5°C for 3.5 min with hydrogen peroxide, 55.6°C for 6.2 min, or 56.7°C for 3.5 min, resulted in a less than 3-log unit reduction of viable *Salmonella* spp. and a less than 0.5-log unit reduction of *L. monocytogenes.* At 53.2°C with peroxide, plate pasteurization resulted in a 3.44-log unit reduction of *S. senftenberg* in 3.5 min. At 57.7°C with no peroxide, the D-value for *Salmonella* spp. was 0.78 min when heated in submerged vials, and plate pasteurization reduced viable numbers by 3.64 log units in 3.5 min. Destruction of *Listeria* under these conditions was still less than 1 log unit. Variation in the pH of the egg white from 7.8 to 9.3 resulted in D-values for *Salmonella* spp. at 56.6°C of 3.60 min to 1.08 min, respectively. D-values for *L. monocytogenes* under these conditions ranged from 10.4 min at pH 7.8 to 20.9 min at pH 9.3. The reduced heat sensitivity of *Salmonella* spp. at lower pH values should be considered in reevaluating pasteurization procedures.

Key words: Egg white, heat resistance, pathogens, *Salmonella, L. monocytogenes*

Pasteurized liquid egg white is used in a variety of processed foods such as baked goods, confections, and chilled or frozen dessert products. Pasteurization procedures were developed *(2, 5, 13, 14, 16)* to ensure that these products are free of *Salmonella* spp. contamination. Egg white presents a particularly difficult problem for the production of pathogen-free pasteurized products. Loss of desired functional properties occurs at temperatures as low as 48.9°C when the time of holding is 30 min or longer; the loss becomes very rapid at 58.9°C *(20).*

Salmonella enteritidis infections have increased substantially in recent years, particularly in the New England and Mid-Atlantic states; the number of outbreaks increased from 26 in 1985 to 67 in 1990 *(3).* Humphrey *(10)* and Humphrey et al. *(11)* suggested that *S. enteritidis* might be more heat resistant than other salmonellae commonly associated with egg products, and Humphrey et al. *(12)* demonstrated that the albumen and the outside of the vitelline (yolk) membrane are the principal sites of contamination with *S. enteritidis* in naturally infected eggs.

In recent years, outbreaks of listeriosis have proven that *Listeria monocytogenes* is an important food-borne pathogen. Its presence in egg products has been demonstrated *(15),* and, although no outbreaks of listeriosis have been attributed to eggs, the potential exists for survival and growth of *L. monocytogenes* in egg products. Sionkowski and Shelef *(19)* found that numbers of *L. monocytogenes* decreased in raw egg albumen stored at 5°C, and that this decline was markedly affected by pH. In heat-treated albumen stored at 5°C, numbers declined initially but then increased rapidly.

The pH of egg white is known to affect the heat sensitivity of *Salmonella* spp. Cotterill *(4)* demonstrated that *S. senftenberg, S. orienburg,* and *S. anatum* became progressively more heat resistant as the pH was decreased from 9.3 to 8.5. Garibaldi et al. *(7)* showed that *S. typhimurium* TM-1 and *S. senftenberg* 775W were more heat resistant at pH 6 or 7 than at pH 8.9.

Changes in the egg industry since the publication of the egg pasteurization procedures in 1969 have been substantial. At the time of development of the pasteurization parameters, commercially broken egg white typically had a pH of 8.9 to 9.1 *(1).* As the processed-foods industry has grown, production of pasteurized liquid egg products has increased. In vertically integrated operations, eggs now may move from the laying house to the breaking operation in a matter of hours instead of days or weeks. As a result, the pH of commercial liquid egg white is frequently at or below 8.2. These developments prompted the USDA Agricultural Mar-

keting Service to establish a cooperative research program to better define the processing requirements needed for thermal inactivation of *S. enteritidis* and *L. monocytogenes*. This study focuses on the heat resistance of these pathogens in egg white at temperatures between 51.5°C and 57.7°C and pH values between 7.8 and 9.3, and also on the influence of H_2O_2 on their thermal resistance.

MATERIALS AND METHODS

Cultures

For all submerged-vial studies, a mixture of *Salmonella* strains and a separate mixture of *Listeria monocytogenes* strains was used. The *Salmonella* mixture included four strains of *S. enteritidis* and one strain each of *S. senftenberg* (not 775W) and *S. typhimurium*. Sources of these strains are as follows: *S. enteritidis* 2000 from Isabel Walls (Eastern Regional Research Center, U.S. Department of Agriculture, Wyndmoor, PA); *S. enteritidis* 5–19 (from yolk sac of a dead chick), Y8P2 (from yolk), and 92-008 (environmental isolate) from Charles Benson (Univ. of Pennsylvania School of Veterinary Medicine, New Bolton, PA); *S. senftenberg* Pro 168 Grp from C. Benson; and *S. typhimurium* from I. Walls. Cultures were maintained individually in tubes of tryptose phosphate broth (TPB) (Difco Laboratories, Detroit, MI) at 5°C. For heating studies, the strains were grown individually in 50 ml of TPB in a 250-ml Erlenmeyer flask on a rotary shaker (37°C, 22 h, 150 rpm). Six-strain mixtures of *Salmonella* were prepared by blending equal volumes of cultures of each organism immediately before use. *S. senftenberg* was used in the plate-pasteurization studies.

The origins of the five *L. monocytogenes* strains used in the mixture are as follows: Scott A (clinical isolate, originally from J. Hunt, FDA, Cincinnati, OH), 2284 (isolated from chicken breast) and Scott A 2045 (both from Sharon Franklin, ARS, Ames, IA), ST.L. and V-7 (Edward Hoerning, USDA, Gastonia, NC). Cultures were carried individually in tubes of BHI broth (Difco) at 5°C. For heating studies, each strain was grown individually in 50 ml of BHI with 0.3 g/liter additional glucose in a 250 ml Erlenmyer flask on a rotary shaker (37°C, 24 h, 150 rpm). Five-strain mixtures were prepared by combining equal volumes of *L. monocytogenes* cultures immediately before use. *Listeria innocua* 2430, isolated from commercial raw egg, was obtained from S. Franklin and was used for plate pasteurization studies.

Heating menstruum

Commercially broken raw egg white was obtained from local egg processors for plate pasteurization and submerged-vial experiments in which heating temperature was the variable under investigation. For submerged-vial studies, egg white was shipped cold in sterile 8 oz containers and arrived at the laboratory within 24 h of breaking of the eggs. The temperature of samples on arrival was 7 ± 2°C. Aerobic plate counts were determined on each batch of egg white received, as described below. Unpasteurized egg white for plate pasteurization was obtained in 30 lb. pails and used within 2 h. For studies of the effect of pH of egg white, shell eggs were obtained within 24 h of laying and held at 5°C in the shell for 0 to 22 days to obtain egg white with pH values of 8.2, 8.8, and 9.3. The eggs were hand-broken, separated, and blended until homogeneous by passing repeatedly through a metal screen. For a pH of 7.8, the pH was adjusted with 0.1 N HCl (Mallinckrodt; Paris, KY); approximately 7 ml/100 g of egg white was required.

Heating procedure

Samples were inoculated to 8.5 to 9.0 log CFU/g in unheated product to ensure that the pathogen being tested greatly outnumbered the natural background flora. This method allowed enumeration on nonselective media with inconsequential interference by the background flora.

For submerged-vial studies, egg albumen (4.5 g) was weighed into a 9-ml glass vial (15 mm o.d. by 60 mm high), 0.5 ml of culture added, and the vial closed with a rubber septum and plastic lid. The contents were mixed, tempered to 25°C, and the vial submerged in a controlled-temperature water bath (Lauda, model MS-20; GMBH & Co KG, Konigshifen, Germany). Heating temperatures were 55.5, 56.6, and 57.7°C without added hydrogen peroxide and 51.5 and 53.2°C with added hydrogen peroxide. The temperature was monitored with a thermocouple (Type T, Omega Engineering, Inc., Stamford, CT) inserted into the geometric center of a control vial containing 4.5 grams of uninoculated albumen and 0.5 ml of peptone water. The temperature was recorded with an Omega Recorder, Series RD-2000 (Omega Engineering, Inc., Stamford, CT). At intervals, vials were removed from the heating bath and cooled immediately in a slush ice bath. Time intervals were 30 s for the first 3.5 min, then 2 or 3 min thereafter depending on the death rate expected. For variables requiring addition of hydrogen peroxide, the vials were removed from the water bath when samples reached pasteurizing temperature and 44 µl of a 10% solution of H_2O_2 (Fisher Scientific; Fair Lawn, NJ) added with a multichannel pipetter. The vials were resealed, the contents mixed by shaking, and the vials returned to the water bath. Timing was initiated when the sample temperature returned to the desired experimental temperature. All experiments were conducted in triplicate. Survival of the background flora was determined by the same procedure as for inoculated samples.

For plate pasteurization studies, unpasteurized albumen was inoculated with single-strain cultures of *S. senftenberg* or *L. innocua* at the same level (8 to 9 log CFU/g) as in the submerged vial studies. An APV Crepaco Junior Plate Pasteurizer (APV Crepaco, Chicago, IL) was used to heat inoculated albumen at 56.6°C and 57.7°C for 3.5 min. Inoculated egg white also was pasteurized at 51.5°C and 53.2°C for 3.5 min with 10% hydrogen peroxide solution injected prior to the beginning of the holding tube at a rate of 0.875 lb/100 lb (ca. 0.397 kg/45.4 kg) of egg albumen. A calibrated delivery system with a Masterflex peristaltic pump (LS Quick Load head 7021-24, drive 7553-30, Cole-Palmer Instrument Co., Niles, IL) and neoprene tubing (1.6 mm i.d.) was used to deliver hydrogen peroxide through an injection port placed at a right angle to the direction of flow; this injection port was immediately followed by two right angles in the flow of product to achieve maximum mixing. The temperature was measured at the end of the holding tube. Samples of unheated and heated products were immediately placed in an ice bath. Three trials were conducted for each organism; each temperature was used in each trial. Replicates at each temperature represent different batches of egg white and culture. Survival of the background flora was determined by the same procedure as for inoculated samples.

Bacteriology

The number of surviving microorganisms was determined by surface plating onto tryptic soy agar (Difco) using a Spiral plater (Model D; Spiral Biotech, Bethesda, MD). The cooled egg albumen (1.1 g) was weighed into sterile tubes, 9.9 ml of sterile 0.1% peptone (Difco) water added and the mixture shaken. Further dilutions were prepared in peptone water as needed. Colonies of surviving *Salmonella* spp. were counted after 24 h at 37°C; surviving *Listeria* spp. were counted after 48 h at 37°C.

Data analysis

Counts were transformed into log values. The data were then analyzed by linear regression using a commercial spreadsheet program. D-values were calculated as the negative reciprocal of the slope of the survivor curve. The correlation coefficient (r^2) for all *Salmonella* trials was 0.95 ± 0.03 and for *L. monocytogenes* trials was 0.90 ± 0.08, indicating that the survivor plots (log number versus time) were linear, and D values could be calculated from the slopes of the lines. All D-values reported are averages of three trials. For albumen with no hydrogen peroxide added, z-values were calculated as the negative reciprocal of the slope of a plot of log D-value against temperature. For plate pasteurization experiments, the reduction in viable count was calculated and compared to the reduction expected from the D-value.

RESULTS AND DISCUSSION

The background flora in samples for survival curves for *Salmonella* spp. (24-h incubation) was 4.96 ± 0.65 log CFU/g and for *L. monocytogenes* (48-h incubation) 5.00 ± 0.70 log CFU/g. Each survival curve consisted of 10 or more data points, only one or two of which were 5 log CFU/g or less. No tailing which could be attributed to heat-resistant microorganisms in the background flora was observed. Therefore we concluded that the background flora did not interfere with calculation of D-values from the survival curves.

D-values for the six-strain mixture of *Salmonella* spp. and the five-strain mixture of *L. monocytogenes* are shown in Table 1. The z-value for *Salmonella* spp. (no peroxide) was 4.03°C (r^2 = 0.9998), while for *L. monocytogenes,* the z-value was 11.3°C (r^2 = 0.8788). Data reported here were obtained without the use of selective media and in commercially broken egg white with a pH of 8.8 or less. Cotterill *(4)* determined the temperature of heating required to give a 4-log unit reduction in 3.75 min (D = 0.94 min), and obtained values of 53.4 to 55.2°C for various species of *Salmonella* in egg white at pH 9.3. Garibaldi et al. *(8),* using *S. typhimurium* Tm-1 in pH 9.2 egg white, obtained D-values of 0.64 min at 54.8°C and 0.25 min at 56.7°C. Kline et al. *(14)* obtained a 7.4-log unit reduction in *S. typhimurium* Tm-1 during 4 min at 134°F (56.7°C); this is equivalent to a D-value of 0.54 min; however, *Salmonella* populations were estimated with a MPN procedure involving the use of selective media, which might have reduced recovery of heat-injured cells. Humphrey et al. *(11)* determined D-values for *S. enteritidis* and *S. typhimurium* in egg white at 55°C and obtained values of 1.5 and 1.0 min respectively; the pH of the egg white was not reported. Palumbo et al. *(18)* assessed the heat resistance of individual strains in our mixture in egg yolk, and found them within the range reported by other workers.

TABLE 1. *Thermal resistance of* Salmonella *spp. and* Listeria monocytogenes *in commercially broken liquid egg white with and without added hydrogen peroxide as determined by submerged-vial technique*

Temperature (°C)	H_2O_2 added (%)	D-value ± SD (min); n = 3	
		Salmonella spp.	*Listeria monocytogenes*
51.5	0.875	3.87 ± 2.09	37.6 ± 17.0
53.2	0.875	1.60 ± 0.25	23.3 ± 8.3
55.5	0	2.74 ± 0.41	13.0 ± 1.6
56.6	0	1.44 ± 0.37	12.0 ± 1.8
57.7	0	0.78 ± 0.09	8.3 ± 1.4

Two processes for pasteurization of egg white with hydrogen peroxide as a processing aid are approved by the USDA *(1):* (i) one in which pH 9 egg white is heated to 125°F (51.7°C) and held for 1.5 min before injecting hydrogen peroxide into the holding tube, and then held an additional 2 min before cooling and adding catalase to decompose residual peroxide; and (ii) another in which hydrogen peroxide solution is injected into pH 9 egg white between the regeneration and heating sections of the heat exchanger, and the product heated to 125°F (51.7°C), held for 3.5 min, and cooled; catalase is added to decompose residual peroxide. Ayres and Slosberg *(2)* demonstrated the effectiveness of hydrogen peroxide plus catalase treatment for destruction of *Salmonella* spp. in egg albumen; their process involved holding egg albumen for 15 or 30 min with 0.05, 0.1, or 0.2% hydrogen peroxide. Later Lloyd and Harriman *(16)* patented a process for combining heat treatment and peroxide use at the natural pH of the egg white. The purpose of adding peroxide 1.5 min into the holding period in method (i) is to allow denaturation of naturally occurring catalase to occur. Since detectable peroxide remained at the end of the heating period in this study, it appears that this goal was accomplished and the data presented here for a heat-plus-peroxide process should be representative of destruction attainable with hydrogen peroxide methods. Heating egg white at 134°F (56.7°C) for 3.5 min or 132°F (55.6°C) for 6.2 min are permitted processes also *(1).*

In order to evaluate the effectiveness of the current processes, the log-unit reduction in viable cell numbers during 3.5 min at each temperature was calculated from the D-values and compared to the actual destruction of *S. senftenberg* and *L. innocua* in the plate pasteurizer (Table 2). Heating at 55.5°C for 6.2 min gave a calculated 2.26-log unit reduction. Plate pasteurization with a 6.2-min holding time was not done. The heat resistance of the *S. senftenberg* and *L. innocua* strains used was determined in egg yolk by Palumbo et al. *(18)* and found to be equivalent to that of the more heat-resistant strains in the *Salmonella* and *L. monocytogenes* mixtures. Heating uninoculated egg white for 3.5 min at 56.6°C reduced the background flora from 4.52 to 3.38 log CFU/g. The *S. senftenberg* population after the same heat treatment was 6.60 log CFU/g; the background flora therefore was not detected on inoculated plates because of the dilution factor. More rapid destruction was achieved in the plate pasteurizer than in submerged vials when hydrogen peroxide was used. We believe this greater rapidity may be attributed to better mixing achieved as a result of turbulent flow in the right-angle turns of the holding tube of the pasteurizer. None of these treatments, however, resulted in better than a 4.5-log CFU/g reduction of *Salmonella* or a 0.8-log reduction of *L. monocytogenes* or *L. innocua.*

The effect of the pH of the egg white on thermal

TABLE 2. *Comparison of log-unit reductions of* Salmonella senftenberg *and* Listeria innocua *in commercially broken egg white after 3.5 min of heating at indicated temperatures, on the basis of calculated D-values from sealed-vial study and actual plate pasteurization*

Temperature (3.5 min, °C)	H_2O_2 added (%)	Reduction ± SD (n = 3) (log CFU/g egg white)			
		S. senftenberg		*L. innocua*	
		Calculated	Plate pasteurization	Calculated	Plate pasteurization
51.5	0.875	0.9	1.80 ± 1.04	0.09	0.48 ± 0.14
53.2	0.875	2.3	3.44 ± 1.00	0.15	0.80 ± 0.38
56.6	0	2.4	2.83 ± 0.24	0.3	0.33 ± 0.21
57.7	0	4.5	3.64 ± 1.03	0.4	0.58 ± 0.22

resistance was markedly different for the two pathogens studied (Table 3). The D-value at 56.6°C for *L. monocytogenes* was 2.6 times greater at pH 9.3 than at pH 7.8. The most likely explanation for this decreased heat resistance at the lower pH is the effect of pH on the rate of heat denaturation of the enzyme lysozyme. Egg white lysozyme has been shown to have antibacterial activity against *L. monocytogenes (9, 21)* and to be more heat-stable at pH 7 than at pH 9 *(5)*. Wang and Shelef *(21)* investigated the effect of heat on the antilisterial effect of raw egg albumen, and found that the effect was progressively lost as the temperature of heat treatment of the albumen increased from 50°C to 80°C.

The thermal resistance of *Salmonella* spp. on the other hand, was almost three times greater at pH 7.8 than at 9.3. The importance of the pH of the egg white in achieving *Salmonella*-free pasteurized product has been previously recognized. Cotterill *(4)* studied the heat resistance of three different strains of *Salmonella* spp. in egg white at pH values between 8.5 and 9.4 (pH adjusted with NaOH or lactic acid). The temperature required to achieve 99.99% reduction in viable numbers (F_m) was determined for three strains; for *S. oranienburg*, F_m increased from 55.0°C at pH 9.4 to 58.6°C at pH 8.5. It is recognized, however, that lactic acid is more bactericidal than HCl and these results may underestimate the effect of natural variation in egg white pH. Garibaldi et al. *(7)* determined D-values at 52.5°C for *S. typhimurium* in egg white adjusted to pH values from 5 to 9 with HCl or NaOH. The D-value at pH 7 was 4.6 times greater than at pH 9.

TABLE 3. *Effect of pH on thermal resistance of* Salmonella *spp. and* L. monocytogenes *in egg white at 56.6°C, showing D-values determined by submerged-vial technique and calculated log-unit reduction in 3.5 min*

pH	*Salmonella* spp.		*Listeria monocytogenes*	
	D-value ± SD[a] (min)	3.5-min reduction (log CFU/g egg white)	D-value ± SD (min)	3.5-min reduction (log CFU/g egg white)
7.8	3.60 ± 0.35	0.97	10.4 ± 2.9	0.34
8.2	2.14 ± 0.43	1.64	16.5 ± 2.6	0.21
8.8	1.59 ± 0.25	2.20	20.3 ± 2.9	0.17
9.3	1.08 ± 0.15	3.24	20.9 ± 2.8	0.17

[a] Three trials, average ± standard deviation.

Data presented here indicate that an increase in temperature from 51.5°C to 53.2°C with hydrogen peroxide and an increase either in time at 56.6°C or in temperature at 3.5 min holding time is needed if the goal of 99.99% destruction of salmonellae is to be achieved. Destruction of *L. monocytogenes* with heat alone may not be feasible. Utilization of the antilisterial activity of naturally occurring lysozyme might be feasible if a holding step is introduced into the process; the activity of lysozyme in pasteurized egg white will depend on the pH of the white as well as the time and temperature employed for pasteurization. Erickson and Jenkins *(6)* inoculated commercially pasteurized liquid egg white with a 5-strain mixture of *L. monocytogenes* and observed little or no change in numbers during storage at 2°C or 6.7°C for the first 10 days; longer storage produced a sharp decline. Sionkowski and Shelef *(19)* found that numbers of *L. monocytogenes* decreased in raw egg albumen stored at 5°C, and that this decline was markedly affected by pH.

Early workers *(17)* stressed the need to keep the initial count of *Salmonella* as low as possible in order that pasteurization could reduce those numbers to negligible levels. Humphrey et al. *(12)* demonstrated that temperature and time of storage of shell eggs were critical in determining the growth of *S. enteritidis* PT4 in egg albumen next to the yolk. Monitoring of pH and adjustment of processing times or pH have also been previously recommended *(4)*. It is still imperative that good manufacturing practices be followed to ensure that the 3- to 4-log unit reduction which can be achieved by heat treatment will be sufficient.

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